REF 30 418

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VIDAS® Testosterone (TES)

IVD

VIDAS Testosterone is an automated quantitative test for use on the VIDAS instruments for the enzyme immunoassay measure of total testosterone in human serum or plasma (lithium heparinate), using the ELFA technique (Enzyme Linked Fluorescent Assay). It is intended as an aid in the diagnosis and management of conditions involving excess or deficiency of this androgen.

SUMMARY AND EXPLANATION

Testosterone, which is the main androgen hormone in men, is secreted by the Leydig cell of the testis, under the influence of a hypophysial hormone called LH (luteinizing hormone). This androgen can have an effect on spermatogenesis, maturation of external genitalia, secondary sex characteristics (beard, pubes and axillary hair) and protidic anabolism.
In women, testosterone is secreted in small quantities by the ovaries and adrenal glands, and is the result of peripheral conversion of precursors (notably androstenedione).
When circulating, testosterone is bound to carrier proteins, principally SHBG (Sex Hormone-Binding Globulin) and albumin. Free testosterone represents 1 - 2% of the total concentration of testosterone (5).

Monitoring of testosterone levels with VIDAS Testosterone is useful:
- in the male, for evaluating steroid secretion of testis. Low concentrations of testosterone are often associated with hypogonadism or feminization of the body.

- in the female, for exploring androgenic secretion. High concentrations of testosterone can be associated with polycystic ovary syndrome, ovarian or adrenal tumor, adrenal hyperplasia or idiopathic hirsutism (1, 2, 3, 4, 6, 7, 8, 9).

PRINCIPLE

The assay principle combines an enzyme immunoassay competition method with a final fluorescent detection (ELFA).
The Solid Phase Receptacle (SPR), serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and pre-dispensed in the sealed reagent strips.
All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.
The sample is taken and transferred into the well containing the conjugate which is an alkaline phosphatase-labeled testosterone derivative. The testosterone present in the serum and the testosterone derivative in the conjugate compete for the anti-testosterone specific antibody sites coated to the inner surface of the SPR. Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The intensity of the fluorescence is inversely proportional to the concentration of testosterone present in the sample.
At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

CONTENT OF THE KIT - RECONSTITUTION OF REAGENTS (30 TESTS) :

30 TES strips	STR	Ready-to-use.
30 TES SPRs 1 x 30	SPR	Ready-to-use. SPRs sensitized with polyclonal anti-testosterone immunoglobulins (rabbit).
TES control 1 x 2 ml (lyophilized)	C1	Reconstitute with 2 ml of distilled water. Wait for 5 to 10 minutes. Mix. After reconstitution, stable 2 weeks at 2-8°C or until the expiration date on the kit when stored at - 25 ± 6°C. 5 freeze / thaw cycles are possible. Human serum* + testosterone + preservatives. Range in ng/ml is indicated on vial label.
TES calibrator 1 x 3 ml (lyophilized)	S1	Reconstitute with 3 ml of distilled water. Wait for 5 to 10 minutes. Mix. After reconstitution, stable 2 weeks at 2-8°C or until the expiration date on the kit when stored at - 25 ± 6°C. 5 freeze / thaw cycles are possible. Human serum* + testosterone + preservatives. Concentration in ng/ml is indicated on vial label.
1 MLE card		Specifications sheet containing the factory master calibration data required to calibrate the test.
1 Package insert		

* This product has been tested and shown to be negative for HBs antigen and antibodies to HIV1, HIV2 and HCV. However, since no existing test method can totally guarantee their absence, this product must be treated as potentially infectious. Therefore, usual safety procedures should be observed when handling.

The SPR

The SPR is coated during production with polyclonal anti-testosterone immunoglobulins (rabbit). Each SPR is identified by the "TES" code. Only remove the required number of SPRs from the pouch. **Make sure the pouch is well closed after opening.**

The Reagent Strip

The strip consists of 10 wells covered with a labeled foil seal. The label comprises a bar code which mainly indicates the assay code, kit lot number and expiration date. The foil of the first well is perforated to facilitate the introduction of the sample. The last well of each strip is a cuvette in which the fluorometric reading is performed. The wells in the center section of the strip contain the various reagents required for the assay.

Description of TES Reagent Strip

Wells	Reagents
1	Sample well.
2 - 3 - 4	Empty wells.
5	Conjugate : Alkaline phosphatase-labeled testosterone derivative + calf serum + gelatine (porcine) + releasing agent + 0.9 g/l sodium azide (400 µl).
6	Empty well.
7 - 8	Wash buffer: Tris-NaCl (0.05 mol/l) pH 7.4 + 0.9 g/l sodium azide (600 µl).
9	Wash buffer: diethanolamine* (1.1 mol/l or 11.5 %) pH 9.8 + 1 g/l sodium azide (600 µl).
10	Reading cuvette with substrate : 4-Methyl-umbelliferyl-phosphate (0.6 mmol/l) + diethanolamine** (DEA) (0.62 mol/l or 6.6 %) pH 9.2 + 1 g/l sodium azide (300 µl).

*** HARMFUL reagent :**
- **R 48/22** Harmful : danger of serious damage to health by prolonged exposure if swallowed.
- **R 41 :** Risk of serious damage to eyes.
- **S 26 :** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- **S 46** : If swallowed, seek medical device immediately and show this container or label.

****IRRITANT reagent :**
- **R 36 :** Irritating to eyes and skin.
- **S 26 :** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

For further information, refer to the Safety Data Sheet available on request.

EQUIPMENT REQUIRED BUT NOT PROVIDED

- Pipette with disposable tip calibrated to dispense 200 µl.
- Powderless, disposable gloves.

WARNINGS AND PRECAUTIONS

- **For *in Vitro* Diagnostic Use only.**
- **For professional use only**
- **This kit contains products of human origin. No known analysis method can totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (see Laboratory biosafety manual - 1993, 2nd edition WHO Geneva).**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- Do not use the SPRs if the pouch is pierced.
- Do not use visibly deteriorated STRs (damaged foil or plastic).
- Do not use reagents after the expiration date indicated on the label.
- Do not mix reagents (or disposables) from different lots.
- Use **powderless** gloves, as powder has been reported to cause false results for certain enzyme immunoassay tests.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.
- The wash buffer (well 9) contains a harmful agent (11.5% diethanolamine). Refer to the risk phrase “R” and the precautions “S” above.
- The optical cuvette with substrate (well 10) contains an irritant agent (6.6% diethanolamine). Refer to the risk phrase “R” and the precautions “S” above.
- Spills should be wiped up thoroughly after treatment with liquid detergent or a solution of household bleach containing at least 0.5 % sodium hypochlorite. See the Operator's Manual for cleaning spills on or in the VIDAS instrument. Do not autoclave solutions containing bleach.
- The VIDAS and mini VIDAS instruments should be regularly cleaned and decontaminated (see the Operator’s Manual).

STORAGE CONDITIONS

- Store the VIDAS TES kit at 2-8°C.
- **Do not freeze SPRs and strips.**
- **Store all unused reagents at 2-8°C.**
- After opening the kit, check that the SPR pouch is correctly sealed and undamaged. If not, do not use the SPRs.
- **Carefully reseal the pouch with the desiccant inside after use to maintain stability of the SPRs and return the complete kit to 2-8°C.**
- If stored according to the recommended conditions, all components are stable until the expiration date indicated on the label. Refer to the kit composition table for special storage conditions.

SAMPLE COLLECTION

Specimen type and collection:

Serum or plasma (lithium heparinate).

It is recommended to validate collection tubes before use as some contain substances which interfere with test results.

Since EDTA causes an increase in the values measured, plasma collected on EDTA should not be used.

Samples containing particles in suspension must be centrifuged before analysis.

None of the following factors have been found to significantly influence this assay :

- hemolysis (after spiking samples with hemoglobin: 0 to 300 µmol/l),
- lipemia (after spiking samples with lipids: 0 to 2 mg/ml equivalent in triglycerides),
- bilirubinemia (after spiking with bilirubin: 0 to 80 µmol/l).

However, it is recommended not to use clearly hemolyzed, lipemic or icteric samples and, if possible, to collect a new sample.

Specimen stability :

If samples cannot be tested on the day of collection, store them at 2-8°C or freeze them (-25 ± 6°C) if the assay cannot be performed within 48 hours. Frozen samples can be stored for a maximum of 2 months (N.B. : slight variances with no clinical consequence may be encountered).

Avoid successive freezing and thawing.

INSTRUCTIONS FOR USE

For complete instructions, see the VIDAS or mini VIDAS Operator's Manual.

Master lot data entry

Before each new lot of reagents is used, specifications (or factory master calibration curve data) must be entered into the instrument (VIDAS or mini VIDAS) using the master lot entry (MLE) card (specifications sheet) included in each kit. If this operation is not performed **before initiating the tests**, the protocol will not run. The master lot data need only be entered once for each lot.

It is possible to enter data automatically using the MLE card or manually.

Calibration

Calibration, using the calibrator provided in the kit, must be performed upon receipt of a new lot of reagents after the master lot data (MLE card) has been entered, and then every 14 days. This operation provides instrument-specific calibration curves and compensates for possible minor variations in assay signal throughout the shelf-life of the kit. A control must be tested after each calibration.

The calibrator, identified by S1, must be tested **in duplicate** (see the Operator's Manual). The calibration value must be within the set RFV (Relative Fluorescence Value). If this is not the case, recalibrate.

Procedure

1. **Only remove the required reagents from the refrigerator and allow them to come to room temperature for at least 30 minutes.**
2. Use one TES strip and one TES SPR from the kit for each sample, control or calibrator to be tested. **Make sure the storage pouch has been resealed after the required SPRs have been removed.**
3. Type or select "TES" to enter the code. The calibrator must be identified by "S1", and tested **in duplicate**. If the control needs to be tested, it should be identified by C1.
4. Mix the samples, the calibrator and/or the control using a Vortex-type mixer.
5. Pipette **200 µl** of sample, calibrator, or control into the sample well.
6. Insert the VIDAS SPRs and strips into the positions indicated on the screen. Check to make sure the color labels with the three letter assay code on the SPRs and the Reagent Strips match.
7. Initiate the assay processing as directed in the Operator's Manual. All the assay steps are performed automatically by the instrument. The assay will be completed within approximately 60 minutes.
8. After the assay is completed, remove the SPRs and strips from the instrument.
9. After the assay is completed, dispose of the used SPRs and strips into an appropriate recipient.

RESULTS AND INTERPRETATION

Once the assay is completed, results are analyzed automatically by the computer. Fluorescence is measured twice in the Reagent Strip's reading cuvette for each sample tested. The first reading is a background reading of the substrate cuvette before the SPR is introduced into the substrate. The second reading is taken after incubating the substrate with the enzyme remaining on the interior of the SPR. The RFV is calculated by subtracting the background reading from the final result. This calculation appears on the result sheet.

The RFV is interpreted by VIDAS.

The results are automatically calculated by the instrument using calibration curves which are stored by the instrument (polynomial model). Results are expressed in ng/ml.

VIDAS Testosterone test is calibrated according to the ID-GCMS technique (Isotope Dilution - Gas Chromatography Mass Spectrometry).

Samples with a testosterone concentration greater than 13 ng/ml must be retested after dilution 1:2 in a serum collected from a female patient (diluent). The concentration of testosterone in the diluent and the dilution factor must be taken into account to obtain the concentration in the sample.

Interpretation of test results should be made taking into consideration the patient history, and any other tests performed.

QUALITY CONTROL

A control is included in each VIDAS Testosterone kit.
This control must be performed immediately after opening a new kit to ensure that reagent performance has not been altered. Each calibration must also be checked using this control. The instrument will only be able to check the control value if it is identified by C1.
Results cannot be validated if the control value deviates from the expected values.

Note

It is the responsibility of the user to perform Quality Control in accordance with any local applicable regulations.

LIMITATIONS OF THE METHOD

Interference may be encountered with certain sera containing antibodies directed against reagent components or substances that affect the reaction. For this reason, assay results should be interpreted taking into consideration the patient history, and any other tests performed.

RANGE OF EXPECTED VALUES

The study was carried out on 247 clinically healthy patients. The following values were found:

Cyclic women :	0.1 - 0.9 ng/ml
Men :	3.0 - 10.6 ng/ml

These values are given for information purposes only.
It is recommended that each laboratory establish its own reference values from a rigorously selected population.

PERFORMANCE

Studies performed using VIDAS Testosterone gave the following results :

Measurement range

The measurement range of the VIDAS Testosterone kit is: 0.1-13 ng/ml.

Detection limit

Defined as the smallest concentration of testosterone which is significantly different from the zero concentration with a probability of 95 % : **0.1 ng/ml.**

Precision

Four samples were tested in duplicate in 40 different runs (2 runs per day) on the same VIDAS.

Within-run reproducibility (intra-assay precision) and reproducibility (total precision) were calculated according to the recommendations of NCCLS Document EP5-T2, volume 12 number 4.

			Within-run reproducibility	Reproducibility
Sample	N	Mean (ng/ml)	CV %	CV %
1	80	0.39	5.32	11.52
2	80	1.86	7.60	11.93
3	80	4.55	2.73	7.80
4	80	9.07	3.61	5.80

Accuracy

Dilution test

Three sera collected from male patients were diluted in a serum collected from a female patient (0.26 ng/ml) and tested singly in three series. The ratio of the mean concentration measured over the mean expected concentration is expressed as a mean recovery percentage.

Serum	Dilution factor	Mean expected concentration (ng/ml)	Mean measured concentration (ng/ml)	Mean recovery percentage (%)
1	1/1	9.71	9.71	100.0
	1/2	4.98	4.98	100.0
	1/4	2.62	2.37	90.6
	1/8	1.44	1.19	82.9
2	1/1	11.07	11.07	100.0
	1/2	5.66	5.60	98.8
	1/4	2.96	2.51	84.7
	1/8	1.61	1.26	78.4
3	1/1	8.09	8.09	100.0
	1/2	4.17	3.95	94.7
	1/4	2.22	1.81	81.8
	1/8	1.24	1.02	82.6

Specificity

Tested compound	Cross-reactivity %
Testosterone	100.00
5 α-dihydrotestosterone	0.98
Δ4-androstenedione	0.07
5 α-androstane-3 α, 17β-diol	0.14
5-androstene-3 β, 17β-diol	0.02
19 nortestosterone (nandrolone)	6.40
11 β-hydroxytestosterone	0.85
Desoxycorticosterone	< 0.01
Corticosterone	< 0.01
Progesterone	< 0.01
SDHA	< 0.01
Estradiol, Estriol, Estrone	< 0.01

Comparison with other test methods

216 serum samples were tested in parallel using the VIDAS kit and a radioimmunoassay kit.

The results are indicated below :
VIDAS = 1.060 RIA – 0.137 with a correlation deviation of 0.966.
Testosterone concentrations of a sample determined using kits from different manufacturers may vary according to the assay and calibration techniques and according to the pathologies and associated treatments.
If a different assay technique is used, and as part of patient follow-up, the laboratory must confirm concentrations obtained with the previous technique.

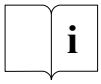
WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. BIRDSALL M. A. and FARQUHAR C. M. Polycystic ovaries in pre and post-menopausal women. Clinical Endocrinology, 1996, 44, 269-276.
2. DELAHUNT J. W. Hirsutism - Practical Therapeutic Guidelines. Drugs, 1993, 45 (2), 223-231.
3. FRANKS, S. Polycystic ovary syndrome. The New England Journal of Medicine, 1995, 333 (13), 853-861.
4. KUTTENN F. and BRICAIRE C. Hyperandrogénie chez la femme. Feuillets de Biologie, 1995, XXXVI (206), 43-48.
5. LITWACK G. Biochemistry of Hormones II : Steroid hormones. In : DEVLIN. T. M., Textbook of Biochemistry with clinical correlations, 3RD ed. Wiley J. and Sons, 1992, 901-925.
6. NATHAN P. Hyperpilosité féminine. Tempo Medical, 1990, 391, 5-11.
7. RITTMASTER R. S. Hirsutism. The Lancet, 1997, 349, 191-195.
8. SOBEL R. and AMICHAI B. Hirsutism. Epidermic distress. The Endocrinologist, 1996, 6 (5), 410-414.
9. VEXIAU P., BOUDOU P., BRERAULT J.L., VILLETTE J.M., JULIEN R., CATHELINEAU G. and FIET J. Hyperandrogénie clinique et stérilité. Immunoanal. Biol. Spéc., 1990, 22, 35-40.

INDEX OF SYMBOLS

Symbole	Signification
REF	Catalogue number
IVD	In vitro diagnostic medical device
	Manufacturer
	Temperature limitation
	Use by
LOT	Batch code
	Consult instructions for use
	Contains sufficient for "n" tests

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